Green Synthesis of Zinc Oxide Nanoparticles from Aqueous Extracts of *Citrus sinensis* Peel: Their Characterization and Relevance

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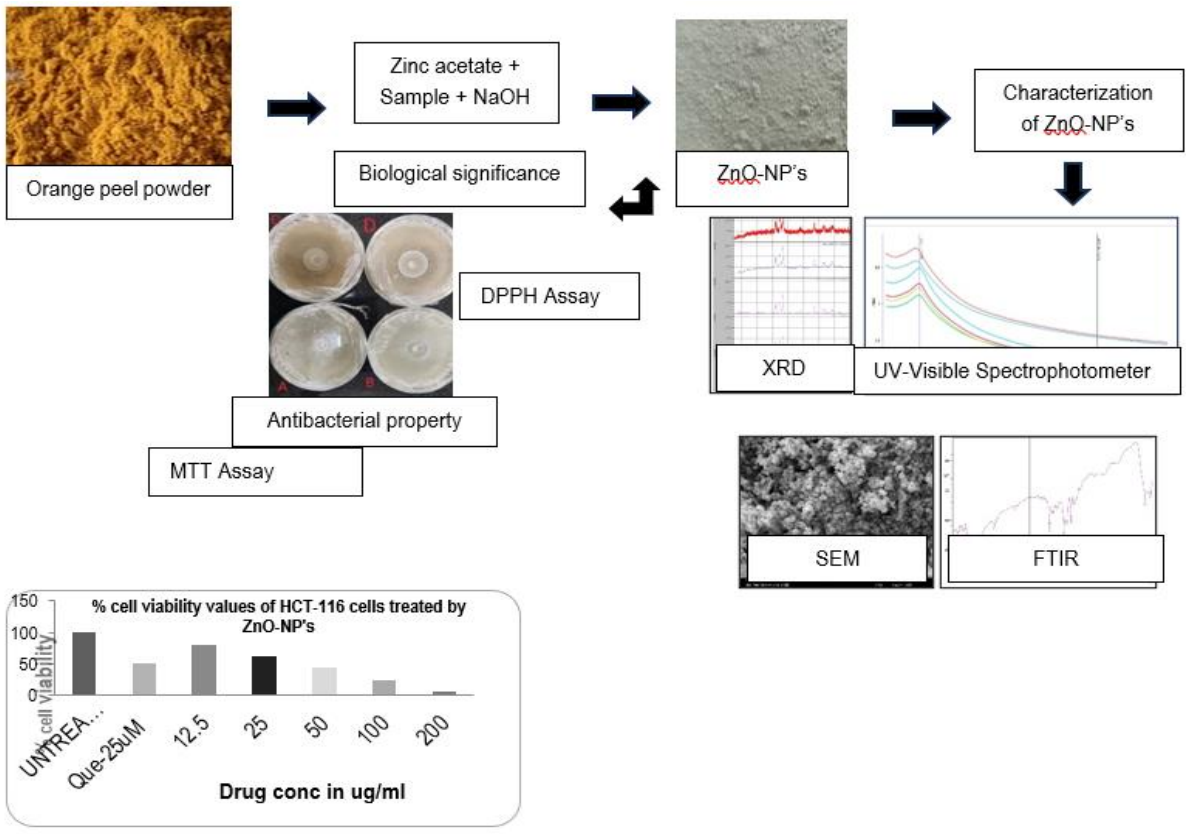
Abstract

Plants and their products serve as a treasure trove of enormous health benefits. The work presents a simple, yet efficient methodology for the green Zinc oxide nanoparticles (ZnO-NPs) synthesis from Citrus sinensis fruit peels. This method seeks to utilize abundantly available agro waste, such as fruit peels, to form value-added biomolecules like minerals and put them into use commercially. The extract from the peels of Citrus sinensis was treated with zinc acetate and sodium hydroxide for the synthesis of ZnO-NPs and was subsequently characterized. Nanocrystal production of ZnO-NPs was determined by XRD and the shape confirmation was determined by SEM analysis. The ZnO-NPs have been studied for their biological importance such as antibacterial and antioxidant properties. An MTT assay was performed to determine the cytotoxic effect on cell proliferation. The XRD analysis provided evidence for nanocrystal formation. The FTIR measurement confirmed the existence of functional groups that contribute to the stability of ZnO-NPs. In the SEM analysis, ZnO-NPs were imaged as well-shaped and spherical.

The nanoparticles were also evaluated for their biological significance for antibacterial, antioxidant and cytotoxic activities. The ZnO-NPs demonstrated that antibacterial action is stronger against Gram-positive bacteria than against Gram-negative bacteria. ZnO-NPs were evaluated for cytotoxicity effect on HCT-116 cells and the IC_{50} value was determined to be 45 µg/ml. These findings can solely be attributed to the well-being of society, as the ZnO-NPs from citrus fruit peels can be used in pharmaceutical applications and can target the reduction of agro waste and as a source of eco-friendly value-added products.

Keywords: *Citrus sinensis,* Green Synthesis, ZnO Nanoparticles, HCT-116 cells.



Graphical Abstract

Introduction

Like any other fruit peel, citrus peels are routinely discarded, regardless of their benefits. Citrus peels possess several functional components and essential oils have been linked to benefit human health as antioxidants and overall protectants against several metabolic illnesses[16]. Most microbial infections, including COVID-19, significantly affect the immune system. Several pharmaceutical companies are striving to develop targeted medicines, vaccines and other vitamin supplements that can boost our immune system. A well-balanced nutritional diet rich in micronutrients such as vitamins and minerals plays a vital role in preserving overall health and preventing chronic infectious diseases.

Micronutrients like zinc, potassium, calcium, chloride and phosphorus may help in overcoming viral disorders[15]. The fruit peels are rich in biomolecules such as minerals which can be extracted and used commercially. Citrus fruit peels contain a high concentration of minerals including iron, copper, manganese and zinc[6]. Every source can be beneficial and can contribute to a healthy society. Several chemical industrial methods are available for the synthesis of zinc as supplements, but they are time-consuming and require labour. This methodology can alleviate some of that difficulty while providing satisfaction from the natural sources. As a result, the goal is to cut costs and to find a low-cost solution [18].

Nanotechnological advancements in several domains across diagnostics, monitoring, tissue engineering, therapy techniques etc., are commercially explored [5]. The green approach for nanoparticle production employs plant resources as reducing and capping agents, which are very promising in nanobiotechnology[4,25]. Compared to other alternative mechanical techniques, this approach is simple, efficient and environmentally friendly. This methodology is also efficient as single-pot reactions, requiring no extra surfactants or capping agents[24]. This strategy also reduces the energy consumed in the processes used to extract zinc salts[19].

In this present investigation, we have attempted to avoid the wastage of natural resources and to target the formation of nano zinc compounds. We describe a green technique for ZnO-NPs utilizing aqueous extracts from *Citrus sinensis* fruit peels. Several methods are used to characterize these nanoparticles, including UV-visible spectroscopy, X-ray Diffraction, Scanning Electron Microscopy and Fourier Transform Infrared Spectroscopy. Various experimental approaches are used to assess the biological significance of ZnO-NPs such as their antibacterial, antioxidant and cytotoxic capabilities.

Material and Methods

C*itrus sinensis* peel extract preparation: Orange peels ready for disposal were collected and dried at 70 °C, converted to a fine powder and sieved using a 250-micron sieve. For later use, they were stored in an airtight container. The 100 ml of deionized water was used to make a 2% solution of orange peel powder and incubated for one hour in a 90 °C water bath. The extract was chilled and filtered. The aqueous filtrate was kept in a freezer at 4°C[9,19].

Green synthesis of ZnO-NPs: C*itrus sinensis* peel aqueous filtrate was added to a zinc acetate solution and heated to 60 °C. The temperature was maintained and stirred continuously. The colour of the extract developed into a pale-yellow colour from colourless. After 5 minutes, 1M NaOH was added and the colour changed instantly from pale yellow to cream white. This provided an early hint of the production of ZnO-NPs. The material was mixed at intervals and absorbance levels were determined using a UV-visible spectrophotometer. The values were taken at one-hour intervals over four hours. The material was centrifuged at 6000 rpm for 20 minutes and then washed for 5 minutes. The washing step was repeated and the nanoparticles were scraped away. The white precipitate was oven-dried and kept in an airtight container[1,20].

Effect of annealing temperature on the green synthesis of ZnO-NPs: To determine the effect of annealing temperature on the formation of ZnO-NPs, they were dried at 300 ºC for 60 minutes in a vacuum furnace and absorbance values were measured in the UV-visible spectrophotometer[10].

Analytical methods for the characterization of ZnO-NPs: The green synthesized ZnO-NPs were studied to determine their optical and nano-structural characteristics. Observing the UV-Vis spectra during the synthesis process allows you to confirm the reduction of pure Zn^{2+} ions. The nanoparticles were dispersed in distilled water and the absorbance values of the samples were measured at intervals. The UV-Visible spectra at wavelengths ranging from 300 to 800 nm were recorded[25]. The morphological characterization of ZnO-NPs involved determining the XRD pattern and examining the crystal structure[8].

Additionally, FTIR was used to identify the functional groups contained in the Zn)-NPs, with spectra obtained in the 400 - 4000 cm^{-1} range. SEM analysis determined the shape and crystallite size of ZnO-NPs extracted from orange peel (SEM JEOL, JSM-IT300) [30].

Determination of antibacterial activity: The antibacterial activity of ZnO-NPs was studied using the well diffusion method. *Bacillus thuringiensis*, *Pseudomonas aeruginosa* and *Escherichia coli* transformant strain DH5 were chosen as the test bacteria. The target microbial culture was spread while the NPs were filled into the wells and allowed to diffuse. The bacteria were swabbed onto Muller Hinton agar medium plates and wells were created and filled with extracted ZnO-NPs in solutions of 0.2 g/ml, 0.4 g/ml, 0.6 g/ml and 0.8 g/ml concentrations in deionized water. The plates were compared with two positive control plates and one negative control plate. The precursor, zinc acetate, was put in the positive control plate at 0.4 g/mL. Deionized water

served as a negative control in the control plate. The zone of inhibition is determined in mm after incubation at 37 °C for 24 hours[20].

Determination of antioxidant activity: The DPPH radical scavenging test was employed to determine the antioxidant property of ZnO-NPs[31]. Ascorbic acid at a concentration of 2 mg/ml was used as a standard and the sample was measured in triplicate. The percentage suppression of absorbance was determined by comparing the samples' antioxidant activity to that of ascorbic acid.

Determination of cytotoxic activity by MTT assay: The MTT colorimetric estimation is one of the popular methodologies for quantifying the viable number of cells in cytotoxicity studies based on the formation of blue formazan by reduction of yellow tetrazolium salt MTT[11]. Living cells produce mitochondrial lactate dehydrogenase enzymes which catalyze the process[2]. The product was detected at 570 nm. Assay controls included medium without cells as a blank, medium with cells but no experimental substance as a negative control and medium with cells treated with 25 µM/ml of quercetin as a positive control. The ZnO-NPs are green synthesized from citrus fruit peels and quercetin, an antioxidant flavonoid present in citrus fruits, was used as positive control[11].

Cell lines and culture media: The HCT-116 has been bought from NCCS in Pune, India. HCT-116 stock cells were cultured in DMEM with glucose and supplemented with 10% foetal bovine serum (FBS), 1% antibiotic–antimycotic solution cultured in a CO_2 cabinet at 37 °C, 5% CO_2, 18 – 20% O_2. The cell line was passaged every 2 – 3 days and 48 passages were carried out. The stock cultures were cultivated in 25 cm^2 culture flasks and all the tests were carried out in 96-well microtiter plates. 200 µl cell solution was planted at 20,000 cells per well and incubated for 24 hours. ZnO-NPs (12.5, 25, 50, 100 and 200 µg/ml) dissolved in DMSO were given to cells and cultured for 24 hours at 37 °C in a 5% CO_2 atmosphere (Table 2).

Following incubation, the spent medium was removed and MTT reagent was added at a final concentration of 0.5 mg/ml for all dosages. A positive (untreated) and negative (quercetin) control was used. The microtiter plate was gently shaken, wrapped with aluminium foil to protect it from light and incubated for three hours. The absorbance at 570 nm was measured using a microtiter reader and the IC_{50} value was computed[1]:

% cell viability = (Mean absorbance of treated cells/Mean absorbance of untreated cells) X 100

Results and Discussion

Researchers use a variety of approaches to achieve green production of various types of nanoparticles. We created ZnO-NPs utilizing an environmentally friendly approach involving *Citrus sinensis* peel (orange peel) extract. When aqueous extracts of *Citrus sinensis* peel were combined with zinc acetate and sodium hydroxide, the color of the mixture shifted from pale orange to dark gray, indicating the first conformation of ZnO-NP synthesis (Fig. 1). The fine powder was collected and stored in an airtight container and set aside for future morphological and physicochemical analysis. Several researchers have standardized the green synthesis types of nanoparticles from agro wastes such as copper nanoparticles from banana and orange peels[21].

Attempts were also made to use vacuum furnace operated at 300 ºC for annealing (Fig. 2). It was found that ZnO-NPs were lighter in color and recovery was considerably low due to splashing in the vacuum chamber (Fig. 3). Some particles recovered from vacuum furnace were subjected to UV-Vis spectrophotometry, as there was no ideal peak seen on the graph; it proved that ZnO-NPs could not be retrieved from the furnace after the thermal processing.

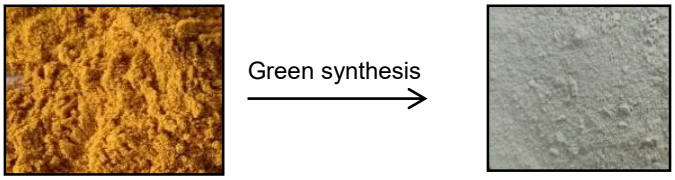


Fig. 1: Dried and powdered orange fruit peels and green synthesized ZnO-NPs

Fig. 2 and 3: Vacuum furnace and Thermal processed nanoparticles

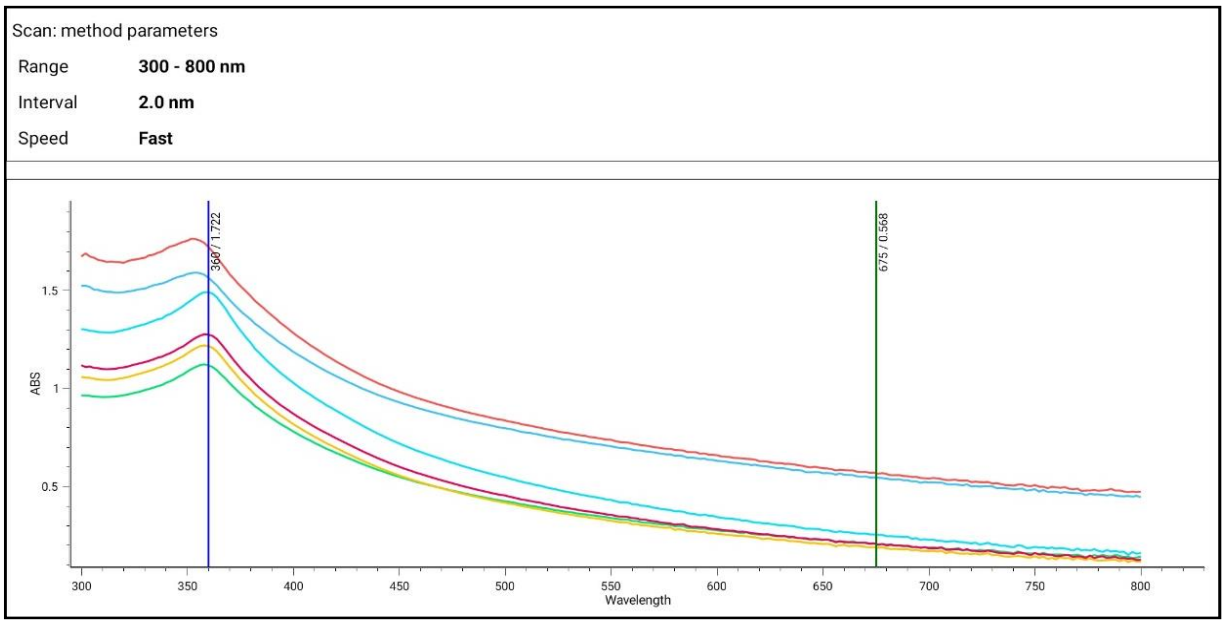


Fig. 4: UV-Visible Spectrophotometer spectrum

Table 1
UV-visible spectrophotometer absorbance peak

Sample Number	Time in hours	Wavelength peak (nm)
1	0.1	350
2	1	352
3	2	358
4	3	360
5	4	360

The decrease in pure Zn^{2+} ions was verified by measuring the UV-visible spectra throughout the process (Table 1). UV-Visible absorption spectra with wavelengths ranging from 300 to 800 nm were used to study the optical characteristics of nanoparticles. They demonstrated a peak shift from 350 nm to 360 nm after 10 minutes and 4 hours (Fig. 4). All absorption peaks agreed with earlier findings in the literature[7]. According to other studies, ZnO-NPs are extracted from three different peels (grape, lime and orange). The ZnO-NPs extracted from orange peel were seen to have high absorbance in the range of 340 - 360 nm[3,9], which shows distinct line broadening of peaks, confirming the existence of nanocrystals.

The diffraction peaks at 2θ at 32.06°, 34.71°, 36.56°, 56.9°, 63.1° and 68.2° indicate the spherical to hexagonal phase of ZnO with crystallinity[9,14,22]. The results revealed that all the distinctive peaks correspond to ZnO-NPs, with no signs of contaminants. FTIR spectroscopy is an analytical tool to identify functional groups in compounds. Figure 6 shows the FTIR spectrum of citrus peel extract-induced ZnO-NPs.

The FTIR spectra revealed multiple peaks for ZnO-NPs at 501 cm^{-1}, 1463 cm^{-1}, 1550 cm^{-1}, 1743 cm^{-1}, 3448 cm^{-1} and 3862 cm^{-1}. The presence of more than five absorption bands indicates complicated development. The peak of ZnO observed at 501 cm^{-1} is typical zinc and oxygen (Zn-O) stretching, which confirms the creation of ZnO-NPs [26]. The characteristic IR frequency range of 1600 and 1500 – 1430 cm^{-1} (strong to weak) corresponds to the C-C stretching vibration of alcohol, carboxylic acid, ether and ester compounds[26]. The absorption peak at 3448 cm^{-1} could be caused by the O-H stretching of hydroxyl groups, which are prominent in organic molecules bound to the surface of nanoparticles and suggest the presence of alcoholic and phenolic chemicals[12,25]. The absorption band between 1700 cm^{-1} - 1800 cm^{-1} indicates the presence of anhydrides.

The ZnO-NPs structural analysis was confirmed by SEM analysis. They were confirmed to be well-shaped and spherical with agglomerates of nanocrystals. Figures 7 and 8 depict SEM images with an EDS graph. Geetha et al[10] obtained a similar finding with SEM analysis. The purity of the ZnO compound was provided by the EDS micrograph.

Biological applications: The antibacterial mechanism of ZnO-NPs, however, is unknown[13]. For determination of antibacterial activity, the plates with Gram-positive bacteria *Bacillus thuringiensis* showed an evident area of inhibition (measured in mm) as shown in figure 9.

Table 2
Cell viability to the respective concentration of ZnO-NPs (MTT assay summary)

Concentration of ZnO-NPs (ug/ml)	% cell viability
Untreated	100
Que-25uM	50.97
12.5	80.64
25	63.13
50	45.21
100	24.67
200	7.24

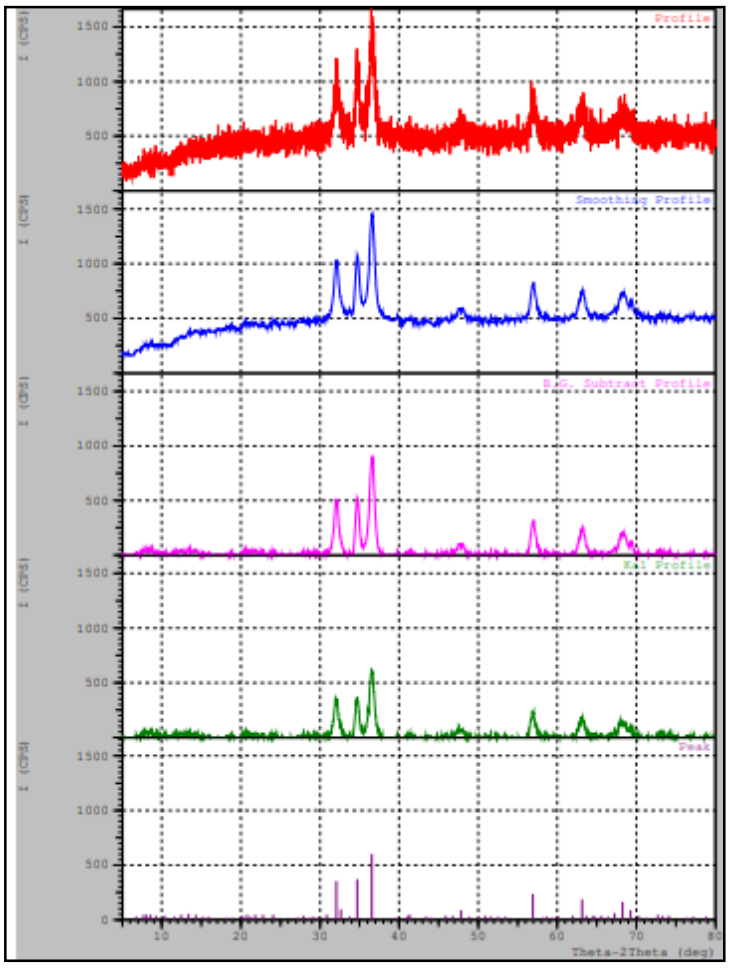


Fig. 5: X-ray diffraction pattern of ZnO-NPs

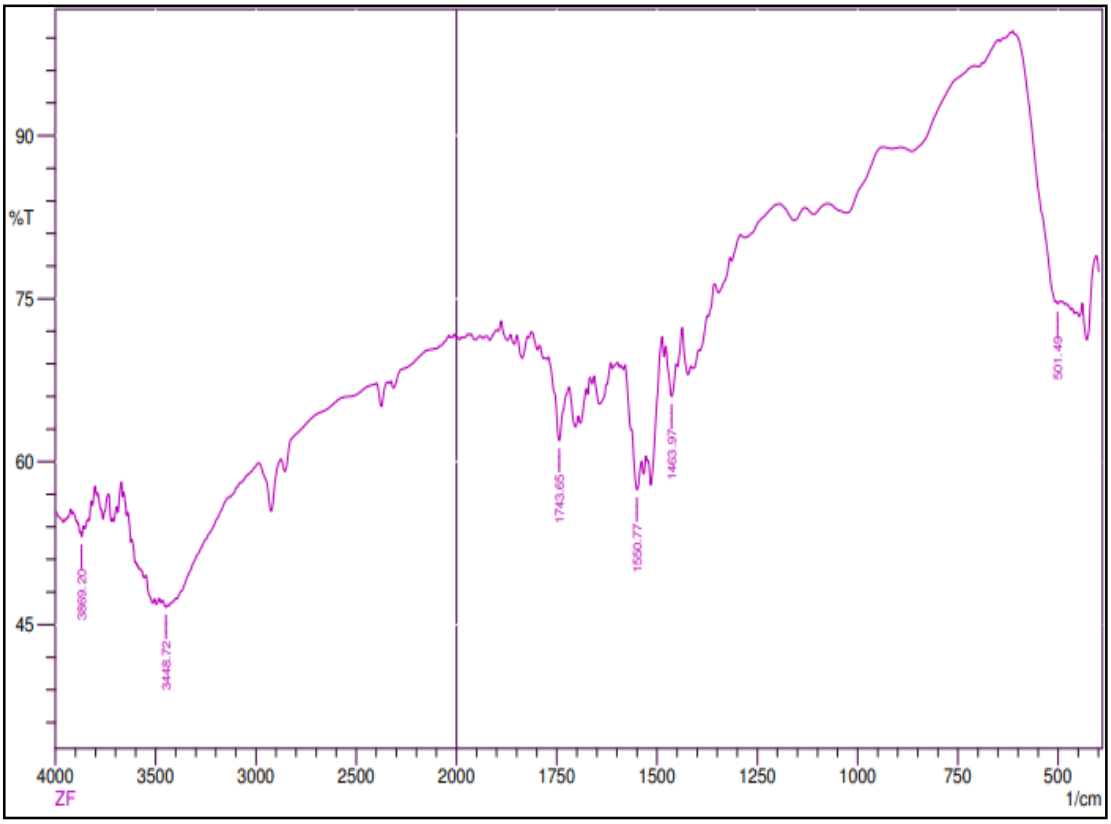


Fig. 6: FTIR spectrum of biosynthesized ZnO-NPs

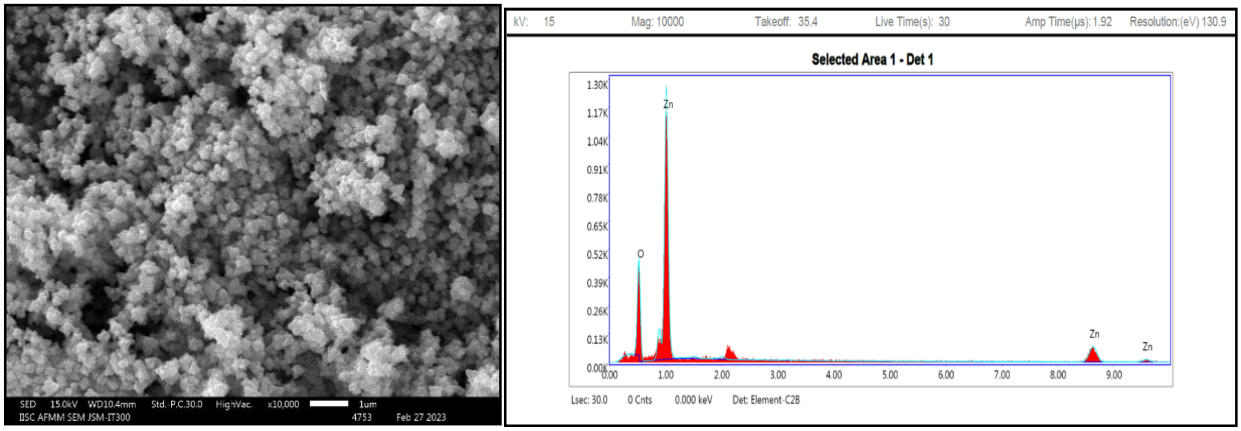


Fig. 7 and 8: SEM image of ZnO-NPs and SEM-EDS

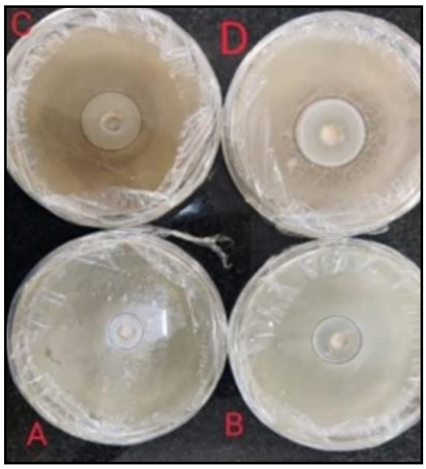


Fig. 9: MHA media plates exhibiting zone of inhibition with varying concentrations of ZnO-NPs
A. 0.2 g/ml – 2 mm zone of inhibition B. 0.4 g/ml – 5 mm zone of inhibition;
C. 0.6 g/ml – 8 mm zone of inhibition D. 0.8 g/ml – 10 mm zone of inhibition

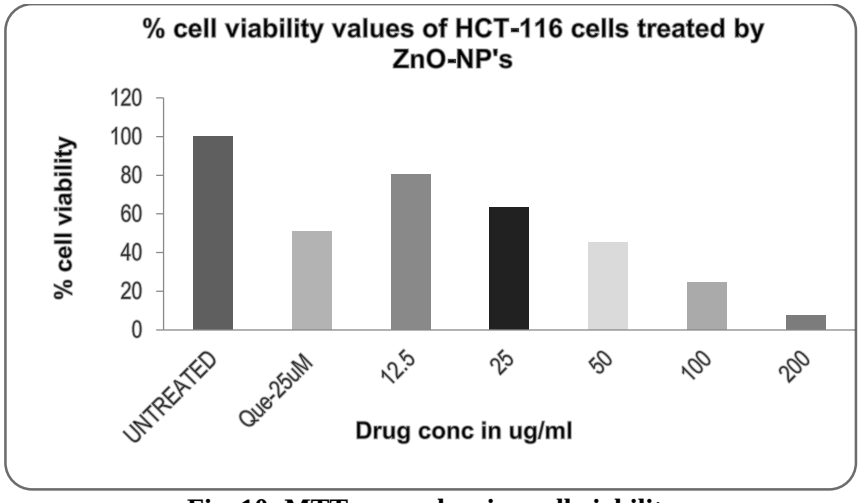


Fig. 10: MTT assay showing cell viability

Both *E. coli* transformant strain and the *Pseudomonas aeruginosa* did not show any effect against the ZnO-NPs[9]. A study by Elkady et al[8] found that the ZnO-NPs showed a strong response against the Gram-negative bacteria like the *Pseudomonas* species, but nanoparticles extracted through other methods showed a major difference in response towards Gram-negative bacterial activity[28,29]. Antioxidant activities of ZnO-NPs were investigated using DPPH radical scavenging activity, which was quantified as ascorbic acid equivalent antioxidant capacity (AEAC). The results suggest that the ZnO-NPs have 1.09 ± 0.03 with good potential as antioxidant nanoparticles in the ascorbic acid standard graph. DPPH is a stable free radical that has a maximum absorbance of 517 nm in ethanol. Radical scavenging measures a decrease in absorption[27]. The cytotoxicity investigation using MTT assay found that ZnO-NPs were

highly harmful to HCT-116 cells, with an IC50 value of 45 µg/ml (Table 2). Quercetin was employed as a standard control. The MTT assay results of ZnO-NPs had substantial cytotoxicity against human colorectal cancer cells with an IC50 value of 45 µg/ml.

Further research is needed to identify the molecular mechanism behind the anti-colon cancer capabilities of the test compounds at the *in vitro* level (Figure 10).

Conclusion

ZnO-NPs synthesized from *Citrus sinensis* peel have multiple benefits and the protocol employed is energy efficient and economical. The nanoparticle was synthesized successfully as observed in the UV-visible spectrum, FTIR and XRD spectrum. Besides, ZnO-NPs were agglomerated as observed under SEM imaging. The green synthesized nanoparticles exhibit antibacterial activity against Gram-positive bacteria and antioxidant activity against DPPH. They were also analysed for cytotoxicity effect on HCT-116 cells and an IC_{50} value of 45 µg/ml was determined.

References

1. Ala A.A., Olotu B.B. and Ohia C.M.D., Assessment of cytotoxicity of leaf extracts of *Andrographis paniculata* and *Aspilia africana* on murine cells *in vitro*, *Archives of Basic Applied Medicine*, **6(1)**, 61–65 **(2018)**

2. Alley M.C., Scudiero D.A., Monks A., Hursey M.L., Czerwinski M.J., Fine D.L., Abbott B.J., Mayo J.G., Shoemaker R.H. and Boyd M.R., Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay, *Cancer Research*, **48(3)**, 589–601 **(1988)**

3. Bajpai S.K., Jadaun M. and Tiwari S., Synthesis, characterization and antimicrobial applications of zinc oxide nanoparticles loaded gum acacia/poly (SA) hydrogels, *Carbohydrate Polymers*, **153**, 60–65 **(2016)**

4. Castro L., Blázquez M.L., Muñoz J.A., González F. and Ballester A., Biological synthesis of metallic nanoparticles using algae, *IET Nanobiotechnology*, **7(3)**, 109–116 **(2013)**

5. Coates J., Interpretation of Infrared Spectra, A Practical Approach, In Encyclopedia of Analytical Chemistry, John Wiley & Sons, Ltd. **(2006)**

6. Czech A., Zarycka E., Yanovych D., Zasadna, Z., Grzegorczyk I. and Kłys S., Mineral Content of the Pulp and Peel of Various Citrus Fruit Cultivars. *Biological Trace Element Research*, **193(2)**, 555-563 **(2020)**

7. Debanath M.K. and Karmakar S., Study of blueshift of optical band gap in zinc oxide (ZnO) nanoparticles prepared by low-temperature wet chemical method, *Materials Letters*, **111**, 116 – 119 **(2013)**

8. Elkady M.F., Shokry Hassan H., Elsayed E. Hafez and Ahmed Fouad, Construction of zinc oxide into different morphological structures to be utilized as an antimicrobial agent against multidrug-resistant bacteria, *Bioinorganic Chemistry and Applications*, doi: 10.1155/2015/536854 **(2015)**

9. Faisal S., Jan H., Shah S.A., Shah S., Khan A., Akbar M.T., Rizwan M., Jan F., Wajidullah, Akhtar N., Khattak A. and Syed S., Green Synthesis of Zinc Oxide (ZnO) Nanoparticles Using Aqueous Fruit Extracts of *Myristica fragrans*: Their Characterizations and Biological and Environmental Applications, *ACS Omega*, **6(14)**, 9709-9722 **(2013)**

10. Geetha M.S., Nagabhushana H. and Shivananjaiah H.N., Green mediated synthesis and characterization of ZnO nanoparticles using *Euphorbia Jatropa* latex as reducing agent, *Journal of Science: Advanced Materials and Devices*, **1(3)**, 301-310 **(2016)**

11. Ghasemi M., Turnbull T., Sebastian S. and Kempson I., The MTT Assay: Utility, Limitations, Pitfalls and Interpretation in Bulk and Single-Cell Analysis, *International Journal of Molecular Sciences*, **22(23)**, 12827 **(2021)**

12. Hameed H., Waheed A., Sharif M.S., Saleem M., Afreen A., Tariq M., Kamal A., Al-onazi W.A., Al Farraj D.A., Ahmad S. and Mahmoud R.M., Green Synthesis of Zinc Oxide (ZnO) Nanoparticles from Green Algae and Their Assessment in Various Biological Applications, *Micromachines*, **14(5)**, 928 **(2023)**

13. Jiang Y., Zhang L., Wen D. and Ding Y., Role of physical and chemical interactions in the antibacterial behavior of ZnO nanoparticles against *E. coli*, *Materials Science and Engineering: C,* **69**, 1361–1366 **(2016)**

14. Khoshhesab Z.M., Sarfaraz M. and Asadabad M.A., Preparation of ZnO nanostructures by chemical precipitation method, *Synthesis and Reactivity in Inorganic, Metal-Organic and Nano-Metal Chemistry,* **41(7)**, 814–819 **(2011)**

15. Kumar P., Kumar M., Bedi O., Gupta M., Kumar S., Jaiswal G., Rahi V., Yedke N.G., Bijalwan A., Sharma S. and Jamwal S., Role of vitamins and minerals as immunity boosters in COVID-19, *Inflammopharmacology*, **29(4)**, 1001–1016 **(2021)**

16. Liu N., Li X., Zhao P., Zhang X., Qiao O., Huang L., Guo L. and Gao W., A review of chemical constituents and health-promoting effects of citrus peels, *Food Chemistry*, **365**, 130585 **(2021)**

17. Namvar F., Rahman H.S., Mohamad R., Azizi S., Tahir P.M., Chartrand M.S. and Yeap S.K., Cytotoxic Effects of Biosynthesized Zinc Oxide Nanoparticles on Murine Cell Lines, *Evidence- Based Complementary and Alternative Medicine*, **1**, 593014 **(2015)**

18. Nava O.J., Soto-Robles C.A., Gómez-Gutiérrez C.M., Vilchis-Nestor A.R., Castro-Beltrán A., Olivas A. and Luque P.A., Fruit peel extract mediated green synthesis of zinc oxide nanoparticles, *Journal of Molecular Structure*, **1147**, 1–6 **(2017)**

19. Okpara E.C. and Fayemi O.E., Comparative study of spectroscopic and cyclic voltammetry properties of CuONPs from citrus peel extracts, *Materials Research Express*, **6(10)**, 105056 **(2019)**

20. Okpara E.C., Fayemi O.E., Sherif E.S.M., Junaedi H. and Ebenso E.E., Green Wastes Mediated Zinc Oxide Nanoparticles: Synthesis, Characterization and Electrochemical Studies, *Materials*, **13(19)**, 4241 **(2020)**

21. Prasad R., Arbiya B., Shivpriya S.N. and Shweta T., Antimicrobial & Free Radical Scavenging Activities of Green Synthesised Copper Nanoparticles from *Musa acuminata* and *Citrus sinensis* Peel Extracts, *Kristu Jayanti Journal of Core and Applied Biology,* **2(1)**, 37-41 **(2022)**

22. Rajiv P., Rajeshwari S. and Venckatesh R., Bio-Fabrication of zinc oxide nanoparticles using leaf extract of *Parthenium hysterophous* L. and its size-dependent antifungal activity against plant fungal pathogens, *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy,* **112**, 384-387 **(2013)**

23. Raut S., Thorat D.P.V. and Thakre R., Green Synthesis of Zinc Oxide (ZnO) Nanoparticles Using Ocimum Tenuiflorum Leaves, *International Journal of Science and Research*, **4(5)**, 1225-1228 **(2013)**

24. Santhosh P.B., Genova J. and Chamati H., Green Synthesis of Gold Nanoparticles: An Eco-Friendly Approach, *Chemistry*, **4**, 345-369 **(2022)**

25. Sawant S., Bhagwat A. and Mahajan C., Synthesis of Cuprous Oxide (Cu_2O) Nanoparticles – a Review, *Journal of Nano- and Electronic Physics,* **8**, 01035–1 **(2016)**

26. Selvarajan E. and Mohanasrinivasan V., Biosynthesis and characterization of ZnO nanoparticles using *Lactobacillus plantarum* VITES07, *Materials Letters*, **112**, 180–182 **(2013)**

27. Shimada K., Fujikawa K., Yahara K. and Nakamura T., Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion, *Journal of Agricultural and Food Chemistry*, **40**, 945-948 **(1992)**

28. Thi T.U.D., Nguyen T.T., Thi Y.D., Thi K.H.T., Phan B.T. and Pham K.N., Green synthesis of ZnO nanoparticles using orange fruit peel extract for antibacterial activities, *RSC Advances*, **10(40)**, 23899-23907 **(2020)**

29. Vargas-Reus M.A., Memarzadeh K., Huang J., Ren G.G. and Allaker R.P., Antimicrobial activity of nanoparticulate metal oxides against peri-implantitis pathogens, *International Journal of Antimicrobial Agents*, **40(2)**, 135–139 **(2012)**

30. Visnuvinayagam S., Murthy L.N., Jeyakumari A., Parvathy U., Anandan R., Sivaraman G.K. and Ravishankar C.N., Combined effect of zinc oxide nano particle incorporated chitosan for better antimicrobial activity towards wound healing, *Journal of Environmental Biology*, **40(4)**, 691–697 **(2019)**

31. Yen G. and Wu J., Antioxidant and radical scavenging properties of extract from *Ganoderma tsugae, Food Chemistry,* **65(3)**, 375-379 **(1999)**.

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